

PRV

PATENT- OCH REGISTRERINGSVERKET
Patentavdelningen

PTO

18 OCT 2004

10/511471
PCT/SE 03/00655

Intyg Certificate

Härmed intygas att bifogade kopior överensstämmer med de handlingar som ursprungligen ingivits till Patent- och registreringsverket i nedannämnda ansökan.

This is to certify that the annexed is a true copy of the documents as originally filed with the Patent- and Registration Office in connection with the following patent application.



(71) Sökande Gambro Lundia AB, Lund SE
Applicant (s) Fraunhofer Inst für Grenzflächen- und
Bioverfahrenstechnik Fraunhofer-Patentstelle,
München DE

(21) Patentansökningsnummer 0201207-8
Patent application number

(86) Ingivningsdatum 2002-04-23
Date of filing

REC'D: 20 MAY 2003

WIPO PCT

Stockholm, 2003-05-09

För Patent- och registreringsverket
For the Patent- and Registration Office


Lina Oljeqvist

Avgift
Fee

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

PATENT- OCH
REGISTRERINGSVERKET
SWEDEN

Postadress/Adress
Box 5055
S-102 42 STOCKHOLM

Telefon/Phone
+46 8 782 25 00
Vx 08-782 25 00

Telex
17978
PATOREG S

Telefax
+46 8 666 02 86
08-666 02 86

AWAPATENT AB

Kontor/Handläggare

Malmö/Dan Henriksson/CBK

GAMBRO LUNDIA AB

Ansökningsnr

Vår referens

SE-2010598

1

A PROCESS FOR PRODUCTION OF A
REGIOSELECTIVE MEMBRANE

Technical field of the invention

- 5 The present invention relates to a process for production of a microporous affinity membrane having regio-selective affinity for compounds in blood or other biologically active fluids to be removed during purification of blood or said fluids, to a microporous affinity membrane produced by said process, to an adsorption device
- 10 containing such a microporous affinity membrane, and to use of such a microporous affinity membrane.

Background art

- 15 Microporous hollow fibre membranes and flat sheet membranes are examples of microporous affinity membranes having a blood side and a filtrate side. Such membranes are well known for analytical, diagnostic or therapeutic purposes. For example, such microporous hollow fibre membranes and flat sheet membranes are useful for
- 20 the treatment of blood or other biologically active fluids with a view to eliminating undesired compounds therefrom, i.e. in therapeutic apheresis. Microporous hollow fibre membranes are normally composed of a bundle of separate microporous hollow fibres. For detoxification
- 25 of whole blood, e.g. dialysis and plasmapheresis, the membrane bundle is normally potted at each end of a polycarbonate tube fitted with two ports in a shell. The blood is normally extracorporeally pumped through a lumen representing the blood side, of each fibre, and a part of
- 30 the blood plasma penetrates, i.e. is filtrated, through the pores of the fibre wall into an outer compartment representing the filtrate side, surrounding each fibre in the bundle. The concentrated blood containing blood cells, too large to enter the pores, and the remaining
- 35 non-filtered part of blood plasma passes through the

lumen. In a venous blood line the filtrated blood plasma stream is normally added to the non-filtered blood stream and returned to the patient.

With a view to eliminating undesired compounds from the blood, the surfaces and pores of the microporous affinity membranes, e.g. microporous hollow fibre membranes and flat sheet membranes, are provided with activated sites or ligands specific for binding to the undesired blood compounds to be eliminated. Such activated sites or ligands are normally based on or bound to functional groups, e.g. amino, carboxy, or sulfonic acid groups, on the microporous membrane surface. The undesired compounds to be eliminated from the blood are normally toxins of different kinds, e.g. bacterial derived toxins. Further examples of such undesired compounds are presented below.

The lumen surfaces on the blood side of microporous hollow fibre membranes, the surfaces on the blood side of flat sheet membranes, the surfaces of the pores and the surfaces on the filtrate side of such membranes are often provided with such activated sites or ligands, particularly for purification of blood or biologically active fluids.

In blood purification applications activated sites or ligands, e.g. positive amino groups as functional groups for heparin or endotoxin adsorption, on the surface on the blood side of such membranes may activate certain blood constituents, e.g. thrombocytes. In such a case, these blood constituents are activated and/or adhered to the ligands and are significantly reduced from the blood. Such an adhesion is undesired. Other blood constituents, e.g. leucocytes, red blood cells and proteins, may in some extent also be adhered to such ligands or activated sites on the blood side of the membrane.

This undesired activation of blood constituents in such membranes has since long been a great problem, in particular the accompanying undesired elimination of

thrombocytes from the blood. Several attempts have been made to solve this problem to prepare microporous hollow fibre membranes and flat sheet membranes lacking the above-mentioned ligands or activated sites on the blood side of the membrane, but so far only complicated processes requiring large amounts of reaction chemicals and solvents have been found. Moreover, these processes are also expensive, ineffective and not environmental friendly, thereby creating problems highly needed to solve.

WO 80/02805 describes, inter alia, a process for the treatment of and/or removal of undesired compounds from whole blood and a membrane therefor. A biologically activated material is immobilised, i.e. ligands are arranged in the pores, and/or on the surface of said membrane that faces away from said whole blood, i.e. faces the filtrate side of the membrane. Further, processes for immobilising different kinds of biologically active material, i.e. ligands, by treatment with chemicals are disclosed. Thus, an asymmetric immobilisation, i.e. creation of regioselective affinity, is disclosed with a view to avoiding contact between blood corpuscles and the immobilising reagent and, thus, pyrogen and/or anaphylactic reactions.

US-A-5,868,936, WO 97/48483, US-A-5,766,908, and EP-A2-0,341,413 disclose immobilising techniques for attaching ligands to the surface of the pores in hollow fibre membranes.

US-A-6,090,292 discloses an asymmetric dialysis hollow fibre coated with albumin essentially on the side facing away from the blood, i.e. facing the filtrate side.

Plasma treatment is known as an effective method for modification of surfaces. It is, inter alia, used to increase the wettability and thus the adsorption properties of surfaces.

EP-A1-0,683,197, US-A-6,022,902, US-A-5,591,140, and US-A-6,013,789 disclose treatment of a surface with plasma with a view to immobilising certain ligands.

US-A-5,597,456 discloses atmospheric pressure plasma
5 treatment of surfaces of medical devices.

EP-A2-0,695,622 discloses plasma modification of flat porous articles using low pressure plasma treatment.

Summary of the Invention

The object of the present invention is to solve the
10 above problem with defective procedures for production of microporous affinity membranes having regioselective affinity for undesired compounds in blood or other biologically active fluids with a view to avoiding undesired
15 activation of constituents in blood or other biologically active fluids in microporous affinity membranes during the purification treatment of blood or said fluids.

This object is achieved with a microporous affinity membrane, produced by a process for production of a microporous affinity membrane having regioselective
20 affinity for compounds in blood or other biologically active fluids to be removed during purification of blood or said fluids, wherein a microporous affinity membrane substrate having a blood side and a filtrate side is subjected to one or more cycles of plasma ignition in the
25 presence of a gas mixture comprising a functional group containing modifying gas, wherein functional groups are regioselectively bound to pore surfaces of the microporous affinity membrane substrate. In a further process step ligands having affinity for said compounds in blood
30 or said fluids may be bound to the functional groups.

In one embodiment functional groups are also regioselectively bound to surfaces on the filtrate side of the microporous affinity membrane substrate.

The present invention also relates to a microporous
35 affinity membrane produced by said process, to an adsorption device containing such a microporous affinity membrane and to use of such a microporous affinity membrane.

Other objects, features, advantages and preferred embodiments of the present invention will become more apparent from the following detailed description when taken in conjunction with the drawings and the appended claims.

Brief Description of the Drawings

Fig. 1 shows a system comprising a microporous membrane adsorption device with regioselectively functionalized microporous hollow fibre membranes produced according to a preferred embodiment of the present invention and having functional groups with ligands bound thereto. Further, in the right part of Fig. 1 such a membrane is shown in an enlarged cross-sectional view.

Fig. 2a shows outside plasma treatment of a membrane substrate at low pressure.

Fig. 2b shows outside plasma treatment of a membrane substrate at high pressure.

Fig. 2c shows inside plasma treatment of a membrane substrate at low pressure.

Fig. 2d shows inside plasma treatment of a membrane substrate at high pressure.

Fig. 3 shows plasma treatment of a microporous flat sheet membrane substrate according to another embodiment of the present invention.

Detailed Description of Preferred Embodiments

In a preferred embodiment the present invention relates to a process for production of a microporous hollow fibre membrane having regioselective affinity.

In another preferred embodiment the present invention relates to a process for the preparation of a microporous flat sheet membrane having regioselective affinity.

Throughout the application text and the claims the following abbreviations are used.

PES = polyethersulfone
PVP = polyvinylpyrrolidone
PP = polypropylene

DACH = diaminocyclohexane

DETA = diethylenetriamine

ESCA = electrospectroscopy for chemical analysis

PFBA = pentafluorobenzaldehyde

5 sccm = standard cubic centimeter

The term "functional group containing modifying gas" or "modifying gas" used throughout the application text means the gaseous form of the molecule leading to surface
10 modification during gas plasma treatment. In the gas plasma these molecules comprising the functional groups are converted to activated species, i.e. radicals or ions. During the gas plasma treatment there is a surface retention of functional groups resulting in a functional
15 membrane surface, i.e. a membrane with regioselective affinity, having the ability to covalently bind different ligands.

The term "blood" used throughout the application text is intended to cover whole blood and different modifications thereof in which one or more of the constituents thereof have been separated off.
20

The term "other biologically active fluids" used throughout the application text means pharmaceutically useful solutions or pharmaceutical preparations which
25 contain a biologically active component, such as a coagulation factor.

The term "blood side" used throughout the application text means the membrane side on which blood or another biologically active fluid is brought to flow
30 during purification by use of a microporous affinity membrane, i.e. either the outer (shell) side or the inner (lumen) side of a microporous hollow fibre membrane, and any of the both sides of a microporous flat sheet membrane.

35 The term "filtrate side" used throughout the application text means the membrane side on which the filtered part of blood or another biologically active fluid

reaches after having passed through the pores of a microporous affinity membrane, i.e. either the outer (shell) side or the inner (lumen) side of a microporous hollow fibre membrane, and any of the both sides of a microporous flat sheet membrane.

The term "compound in blood..." used throughout the application text means an undesired compound intended to be removed from the blood.

The terms "blood constituent" and "constituents in blood" used throughout the application means components normally existing in blood, e.g. different blood cells and proteins.

The term "gas mixture" used throughout the application text means the mixture between modifying gas and carrier gas, but is also used, for simplicity reasons, for the embodiment when the carrier gas is absent.

The term "gas plasma mixture" used throughout the application means the medium resulting from the plasma ignition of the gas mixture and containing the activated species providing the binding of functional groups to the surfaces in question.

The terms "microporous affinity membrane substrate" and "membrane substrate" used throughout the application text means an untreated, not functionalised microporous affinity membrane, i.e. lacking regioselective affinity and intended as a start material in the process according to the present invention.

The term "microporous hollow fibre membrane" used throughout the application text is intended to cover everything from one microporous single hollow fibre up to several single hollow fibres and one or more bundles of such microporous hollow fibres, each fibre having a filtrate side and a blood side.

The term "microporous flat sheet membrane" used throughout the application text means a micropore containing flat membrane having a filtrate side and a blood side.

In one preferred embodiment of the present invention microporous hollow fibre membranes are regioselectively modified or functionalised only on the outer surface, i.e. the filtrate side, and on the surfaces within the pores in an improved way compared to known techniques. The membrane lumen surface, i.e. on the blood side, which comes into contact with whole blood when the membranes are used for blood treatment in therapeutic apheresis, is to remain unmodified. This is achieved by avoiding affinity on the blood side, thereby inhibiting the interaction between certain blood constituents and the ligands bound to functional groups introduced regioselectively during the membrane modification process. This is an important requirement for selective removal of compounds from whole blood or other biologically active fluids within a membrane adsorption device.

Referring to Fig 1 the right part thereof shows a preferred embodiment of a regioselective microporous hollow fibre membrane for treatment of blood in an enlarged cross-sectional view. The flow of blood is marked with an arrow on the blood side. The membrane wall pores connects the blood side with the filtrate side. The flow of blood plasma containing the compounds to remove from blood is marked with arrows in the pores. On the surfaces of the pores and on the outer surface on the filtrate side functional groups, to which ligands are attached, have been bound. To some of said ligands compounds to be eliminated have been bound. As appears, no functional groups are attached to the lumen surface on the blood side.

A major advantage of the present invention compared to prior art, e.g. WO 80/02805, is that the need for reaction chemicals and solvents is highly reduced and that the total costs, e.g. the cost for disposal of chemicals, is lowered. Moreover, the present invention provides a more environmental friendly process compared to prior art processes for the production of such regioselective

membranes. The present invention does not require any organic solvent or chemicals that needs to be eliminated after the treatment, i.e. the gas mixture used reacts totally and no side products are left to be taken care of afterwards.

Further advantages of the present invention include that the microporous affinity membranes having regio-selective affinity are much easier to manufacture compared to the conventional wet-chemical approaches. This is due to the gas plasma treatment process. Moreover, the present invention provides high versatility in that a variety of different functional groups can be arranged to immobilise compounds to be eliminated. This is possible due to independence of the chemicals used in prior art processes. By means of the gas plasma treatment it is possible to introduce reactivity in almost all molecules as long as the molecules can ignite to plasma, why a wide variety of functional groups may be chosen. Further, high efficiency due to improved mass transport properties is obtained, i.e. convective transport of blood compounds to eliminate, e.g. toxins, to the binding sites, i.e. ligands, compared to the corresponding diffusion transport in affinity columns.

In another preferred embodiment of the present invention a microporous flat sheet membrane having regio-selective affinity is produced with a process corresponding to the process for preparing microporous hollow fibre membranes having corresponding properties. This process is described in detail below, e.g. in Example 3.

The functional groups to be introduced on the membrane substrate surfaces of interest are preferably amino groups originating from such molecules as amino compounds (diamines, triamines), e.g. diaminocyclohexane (DACH) and diethylenetriamine (DETA), preferably diaminocyclohexane, but also from all organic precursors with primary amino groups or mixtures of hydrogen with nitrogen or ammonia, provided their vapour pressure is high enough to give a

substantial amount of the molecule containing the functional groups in the vapour phase. Further, other functional groups than amino groups can be introduced, e.g. carboxyl, hydroxyl, sulfonic acid, ester or epoxy groups, when precursors comprising corresponding functions are used instead of compounds containing amino functions.

The microporous affinity membranes produced according to the present invention are made of a biocompatible polymeric material, e.g. polyethersulfone (PES), polyvinylpyrrolidone (PVP), polypropylene (PP), polysulfone (PSU), polymethylmethacrylate (PMMA), polycarbonate (PC), polyacrylonitrile (PAN), polyamide (PA), polytetrafluoroethylene (PTFE), cellulose acetate (CA), cellulose nitrate or regenerated cellulose.

The inner diameter of the hollow fibres is normally 200-1000 μm , the wall thickness is normally 20-200 μm and the pore diameter 0.1-2.0 μm . The fibres are normally arranged in modules e.g. containing a bundle of 10 to more than 1000 fibres, but single hollow fibres are also possible to treat. Experimental modules contain 10-100 fibres. Final modules for blood treatment contain more than 1000 fibres. Modules with more fibres may also be modified according to this procedure.

According to the present invention the hollow fibres used for the microporous hollow fibre membrane are preferably made of a blend of polyethersulfone and polyvinylpyrrolidone with an inner diameter of 330 μm , a wall thickness of 110 μm and a pore diameter of 0.4 μm .

The flat sheet membrane is preferably made of a mixture of polyethersulfone and polyvinylpyrrolidone with a wall thickness of 20-200 μm , preferably 110 μm , and a pore diameter of 0.1-0.8 μm , preferably 0.4 μm .

As stated above, the regioselective introduction of amino groups, i.e. the preferred functional groups, only on the pore surfaces, in practice gradually less towards the blood side, and on the filtrate side, but not at all on the blood side of the microporous affinity membrane

substrate, is achieved by gas plasma treatment of the membrane substrate, preferably using DACH or DETA, most preferably DACH, as the functional group containing modifying gas, and a stabilising carrier gas, which is chemically inert during the gas plasma reaction. Preferably helium is used as carrier gas due to the wide pressure range used for ignition of gas plasma. The use of low gas plasma power is beneficial with respect to the preservation of the functional groups. As alternative carrier gases nitrogen, hydrogen and argon or corresponding mixtures may be used. A further possibility is to work without any carrier gas. During the gas plasma treatment this mixture of modifying gas and carrier gas includes the activated species described above and provides the regio-selective introduction of the amino groups on the surfaces of interest, however, not on the blood side of the microporous affinity membrane substrate, due to deactivation of activated species on the way from the plasma glow discharge zone to the blood side. The proportion between the functional group containing modifying gas and the carrier gas is normally 1:10 - 1:1, preferably 1:4.

The most important parameters are the direction of the gas plasma mixture flow in relation to the membrane substrate to be treated, the mean free path length of the activated species and the flow rate of the gas plasma mixture.

The ligands to be bound to the functional groups introduced on the surface of the membrane substrate filtrate side and on pore surfaces are chosen dependent on the type of compounds to be removed from the blood or any other biologically active fluid. Examples of ligands are proteins, peptides, amino acids, carboxylic acids, oligonucleotides and mixtures of two or more thereof or any other convenient biomolecules. The ligands are added to the functional groups in a separate wet-chemical process, known per se.

The regiospecific introduction of the functional groups can be achieved in four different ways for microporous hollow fibre membrane substrates, comprising four different embodiments of the process according to the present invention, as appears from Figs 2a-2d, i.e.

- 2a) outside low pressure treatment (diffusion control)
- 2b) outside high pressure treatment (laminar or convective control)
- 2c) inside low pressure treatment (laminar or convective control)
- 2d) inside high pressure treatment (diffusion control)

The processes shown in Figs 2a and 2b represent a first main mode and provide a regiospecific functionalisation of the outer surface and the pore surface; the processes shown in Figs 2c and 2d represent a second main mode and provide a regiospecific functionalisation of the inner surface and the pore surface. Thus, these four different embodiments (or two main modes) are intended for different uses, i.e. depending on if the lumen surface is intended to be on the blood side or the filtrate side of the microporous hollow fibre membrane.

As appears from Fig. 2a, showing one embodiment of outside low pressure treatment, diffusion controlled outside plasma treatment under low pressure (0.1-10 mbar), preferably about 1.6 mbar (0.3 mbar modifying gas)) is performed by adding the gas mixture to the outside of the microporous hollow fibre membrane substrate. A fibre module of a hollow fibre membrane substrate is placed between two electrodes, preferably ring electrodes, around a polycarbonate housing. Openings in the housing allow a gas flow along the outer surface of the membrane substrate. After appropriate evacuation the gas mixture is introduced and ignition is performed creating a gas plasma mixture. The gas plasma mixture penetrates the membrane substrate structure by diffusion, i.e. the driving force from mass transfer equals the concentration gradient. The process preferably involves one to ten

cycles of plasma ignition at 13.56 MHz during 1 to 10 sec under the gas mixture atmosphere, followed by a plasma-off period of 2-3 minutes. During the flow of the gas plasma mixture functional groups, e.g. amino groups, are attached to the outer surfaces and the pore surfaces of the hollow fibre membrane substrate. Finally, the fibre modules are evacuated for 1-60 min, normally about 15 min, to remove non-adsorbed modifying gas present in the gas mixture. The inlet and outlet openings for the gas mixture are preferably located at the opposite ends of the housing. This embodiment gives highly satisfactory results as to regiospecific affinity for a hollow fibre membrane for whole blood treatment and is therefore the most preferred embodiment of the present invention.

As appears from Fig. 2b outside plasma treatment under high pressure (50 mbar - 1.1 bar) is performed in the same way as for the low pressure treatment except for the fact that the gas plasma mixture penetrates the membrane substrate structure by convection or laminar flow.

As appears from Fig. 2c convection or laminar flow controlled inside plasma treatment under low pressure (0.01-50 mbar) is performed by adding the gas mixture into both ends of the fibre bundle, wherein the gas mixture penetrates the pores from the lumen side to the outer side of the hollow fibres, i.e. to the polycarbonate housing space, and then exits the housing space through the gas mixture exits arranged perpendicular or substantially perpendicular to the fibre bundle direction. Further, the electrodes are preferably arranged in such a way that the gas mixture exits are arranged between the electrodes.

As appears from Fig. 2d diffusion controlled inside plasma treatment under high pressure (50 mbar - 1.1 bar) is performed by adding gas mixture at one end of the fibre bundle, wherein the gas mixture exits shown in Fig. 2c are closed and the concentric polycarbonate housing or casing surrounding the fibre bundle is filled with a

blocking fluid, e.g polyethylene glycole, thereby allowing the gas mixture to more or less fill the pores but preventing it from passing out from the pores to the outer surface. Instead, the gas mixture exits the fibre bundle at the opposite end.

In the process for preparation of a microporous hollow fibre membrane according to the present invention, the ignition frequency during the plasma ignition is 1 kHz - 13.56 MHz or multiples of 13.56 MHz or microwave frequency, the power is 0.5-20 W, the voltage of the electrodes is 50-500 volts, the pressure is 0.01-10 mbar, the flow rate is 0.1-200 sccm/min, and the gas plasma mixture flow period is up to 20 min.

The plasma treatment experiments and the analyses described below were carried out, if not otherwise stated, for a microporous hollow fibre membrane having regioselective affinity produced according to the most preferred embodiment according to the present invention, i.e. wherein DACH/helium as gas mixture was added to the membrane substrate during the plasma treatment.

For microporous flat sheet membranes the regioselective introduction of the functional groups is achieved as follows.

Fig. 3 shows the preparation of a microporous flat sheet membrane having regioselective affinity for undesired compounds in blood or other biologically active fluids by use of plasma ignition. The flat sheet membrane substrate is enclosed in a housing or casing, having a first and a second compartment separated from each other by the flat sheet membrane substrate. During the plasma ignition treatment the gas mixture is initially introduced in the first compartment, also comprising a plasma chamber with two electrodes connected to a power supply. After the plasma ignition of the gas mixture the gas plasma mixture obtained flows against and passes the flat sheet membrane substrate perpendicularly arranged in relation to the gas plasma mixture flow. The flat sheet

membrane substrate surface facing the first compartment, i.e. on the intended filtrate side of said membrane substrate, and the pore surfaces are regioselectively provided with functional groups. No functional groups are bound to the flat sheet membrane substrate surface facing the second compartment, i.e. on the intended blood side of the membrane. Excess gas continues to flow through the second compartment and is then evacuated therefrom. A vacuum pump connected to the second compartment provides the flow through the whole arrangement. Appropriate ligands are then bound to the functional groups in a conventional way.

In the process for preparation of a microporous flat membrane according to the present invention, the ignition frequency during the plasma ignition is 1 kHz - 13.56 MHz or multiples of 13.56 MHz or microwave, the power is 1-20 W, preferably about 5 W, the voltage of the electrodes is 50-300 volts, the pressure is 0.1-5 mbar, preferably about 0.3 mbar, the flow rate is 1-100 sccm/min, preferably 10 sccm/min, and the gas plasma mixture flow period is up to 30 min, preferably about 5 min. The parameters during this plasma ignition treatment are further described in Example 3.

It is to be understood that the housings or casings, inlets, outlets, electrodes etc in the devices shown in Figs 2a-2d and 3 may be altered as to size, mutual arrangement, type and geometry, still giving the beneficial effects desired for the present invention.

An electron spectroscopy for chemical analysis (ESCA) was performed with a view to quantitatively evaluating the amino group distribution resulting from the plasma treatment.

First a sample of a microporous affinity membrane with regioselective affinity is illuminated with Al k-alpha rays (1486.6 eV), and the energy of emitted electrons is measured. Fluorine is used only as a marker for the functionality arranged at the membrane surfaces,

which itself does not contain any fluorine. Instead, the membrane surface functionalities are derivatised with a fluorine containing compound, e.g.

5 pentafluorobenzaldehyde, with a view to quantifying the functional groups bound to the membrane surfaces.

The derivatisation procedure is preferred as follows: 300 µl stock solution of 0.1 M PFBA in pentane is added to 15 ml pentane. After addition of the test material the solution is brought to react during 2 hours
10 at 39°C in a water bath and under reflux. This is followed by washing during the night in pentane in a Soxhlet device at 43°C (one cycle: 20 min).

In the table below the distribution of atoms in the functional groups on the outer (shell) and inner (lumen)
15 surface of microporous hollow fibre membranes is shown. It appears that, due to the preferred embodiment of the process according to the present invention, the presence of primary amino groups on the inner surface is zero (no fluorine-signal!). The 1.4 atom% nitrogen is due to the
20 PVP content of the membrane.

Table: Atom distribution (ESCA) of plasma-modified PES/PVP membranes after derivatisation with pentafluorobenzaldehyde

Surface	Distribution of elements [%]				
	C	O	S	N	F
Shell	73.0	10.2	0.7	8.3	7.8
Lumen	74.5	21.7	2.4	1.4	-

25

Further, an ESCA analysis was performed with a PP membrane treated with different plasma treatment modes. The table below shows the atom distribution of plasma treated (DACH) PP membrane substrates on inner (lumen)
30 and outer (shell) surfaces of hollow fibre membranes.

Atom distribution (ESCA) of plasma treated (DACH) PP membranes

N may here be used as marker as PP does not contain N.

5

Treatment mode	Surface	Distribution of elements [%]		
		C	O	N
Gas stream outside, parallel to fibres (diffusion controlled)	shell	90.0	6.9	3.1
	lumen	94.8	5.2	-
Gas stream through the membrane wall of hollow fibre (convection controlled)	shell	84.7	7.2	8.1
	lumen	94.2	4.5	1.3

This indicates an approximate 5-fold surplus of amino groups on the outer surface relative to the inner surface for a convection controlled process and the absence of amino groups on the inner surface for a diffusion controlled process.

Moreover, the table below shows the concentration of introduced active amino groups depending on the treatment mode used to introduce them for a PES/PVP hollow fibre membrane.

15

Treatment mode	NH ₂ concentration [mmol/g]
Outside plasma, low pressure (Fig. 2a)	0.08-0.09
Outside plasma, high pressure (Fig. 2b)	0.03
Inside plasma, low pressure (Fig. 2c)	0.02
Inside plasma, high pressure+blocking fluid (Fig. 2d)	0.06

The highest concentrations are achieved with the outside plasma/low pressure mode treatment (see Fig 2A). These concentrations come close the ones required for the

20

monomolecular immobilisation of peptides of several 1000 Da.

Thus, regioselective modification of membrane substrates with a higher selectivity for the outer surface
5 can be achieved. This makes the membranes interesting for arranging ligands selectively on their surfaces. As stated above the regioselectively arranged ligands enable a selective removal of toxins or other target compounds by adsorption during therapeutic purification of blood or
10 other biologically active fluids, while the interaction of constituents in blood or such fluids with the ligands or adsorbed toxins is avoided.

Examples of compounds of interest to remove from blood or other biologically active fluids are e.g. endo-
15 toxins and inflammatory mediators in septic patients, pathogenic antibodies in several immune diseases, low-density lipoproteins in patients with coronary heart disease and drug resistant hypercholesterolemia, and fibrinogen used for the treatment of microcirculatory disorders.
20

The present invention also relates to use of the microporous affinity membrane produced according to the present invention and having regioselective affinity in therapeutic apheresis, for diagnostic applications when
25 enrichment of trace materials is necessary (e.g. pesticides in food or water, metabolites and drugs in plasma, urine, and saliva), and for drug development applications. Common for these different applications is that blood constituents are not activated during the use
30 of the microporous affinity membrane.

In the following examples of the process according to the present invention, functionalisation, i.e. providing regioselective affinity, with amino groups for a single hollow fibre, a fibre bundle modification and a
35 flat sheet membrane substrate, respectively, is shown for PES-PVP microfiltration membranes.

Example 1: Single hollow fibre modification

The plasma treatment mode shown in Fig 2a) was used. The fibre length was 15 cm and the tube diameter 1.2 cm. The system was evacuated at a pressure below 0.01 mbar during 15 min. DACH was added at a flow rate of 0.5 sccm/min; applicable range: 0.1-200 sccm/min) at a pressure of 0.3 mbar (applicable range: 0.1-10 mbar). The plasma ignition was performed at 13.56 MHz (1kHz to 13.56 MHz) and multiples of 13.56 MHz and microwave at 15 W (applicable range: 0.5-200 W) during 1 sec (applicable range: 0.1 sec - 10 min). After the plasma treatment step the system was flushed with H₂ at 10 mbar during 5 min, followed by venting with N₂ to minimize oxidation of the membrane.

15 Example 2: Fibre bundle modification (50 hollow fibres)

The steps in Example 1 were repeated with the exceptions that 2 sccm/min (applicable range: 1-100 sccm/min) helium was added as carrier gas together with the DACH, that the total pressure was 1.2 mbar (applicable range: 0.1-10 mbar), that the effect at the plasma ignition step was 2 W (applicable range: 1-20 W) and that the plasma time was 15 min (applicable range: 10 sec - 30 min). This parameter set results in proper amino functionalisation of outer surfaces and inner pore surfaces of all 50 hollow fibres.

Example 3: Modification of a microporous flat sheet membrane substrate

The plasma treatment mode according to Fig. 3 was used. The system was evacuated at a pressure below 0.01 mbar. H₂ was added at a flow rate of 10 sccm/min together with DACH at a total pressure of 0.3 mbar. The plasma ignition was performed at 13.56 MHz and an effect of 5 W and the plasma time was 5 min.

The result obtained is a flat sheet membrane regio-selectively functionalised with amino groups on the surface on the filtrate side and on the pore surfaces, but not on the surface on the blood side.

CLAIMS

1. A process for production of a microporous affinity membrane having regioselective affinity for
5 compounds in blood or other biologically active fluids to be removed during purification of blood or said fluids, wherein a microporous affinity membrane substrate having a blood side and a filtrate side is subjected to one or more cycles of plasma ignition in the presence of a gas
10 mixture comprising a functional group containing modifying gas, wherein functional groups are regioselectively bound to pore surfaces of the microporous affinity membrane substrate.

2. The process according to claim 1, wherein a
15 microporous hollow fibre membrane substrate is subjected to the plasma ignition.

3. The process according to claim 1, wherein a microporous flat sheet membrane substrate is subjected to the plasma ignition.

20 4. The process according to any one of the preceding claims, wherein ligands having affinity for the compounds in blood or other biologically active fluids are bound to the functional groups.

25 5. The process according to any one of the preceding claims, wherein the functional groups also are regioselectively bound to surfaces on the filtrate side of the microporous affinity membrane substrate.

30 6. The process according claim 4, wherein the ligands are proteins, peptides, amino acids, carboxylic acids, nucleotides, oligonucleotides, antigens or antibodies, and mixtures of two or more thereof.

35 7. The process according to any one of the preceding claims, wherein the functional group containing modifying gas comprises an amino, aldehyde, ester, epoxy, hydroxi or sulfonic acid group, preferably an amino group.

8. The process according to claim 7, wherein the functional group containing modifying gas is diamino-cyclohexane (DACH) or diethylenetriamine (DETA), preferably diaminocyclohexane.

5 9. The process according to any one of the preceding claims, wherein the gas mixture also contains a carrier gas.

10 10. The process according to claim 9, wherein the carrier gas is any gas which is chemically inert during the process, preferably helium, nitrogen, hydrogen, argon or mixtures thereof, most preferably helium.

11. The process according to any one of the preceding claims, wherein the flow rate of gas plasma mixture obtained by the plasma ignition is 0.1-200 sccm/min.

15 12. The process according to any one of the preceding claims, wherein the proportion between the functional group containing modifying gas and the carrier gas is 1:100 to 1:1, preferably 1:4.

20 13. The process according to any one of the preceding claims, wherein up to 10 cycles of plasma ignitions are performed.

25 14. The process according to any one of claims 2 and 4-13, wherein the microporous hollow fibre membrane substrate is enclosed in a housing or a casing throughout the process, preferably a concentric housing or casing.

30 15. The process according to claim 2 or 14, wherein the gas plasma mixture obtained by the plasma ignition is flowing axially along the outer or inner surface of the microporous hollow fibre membrane substrate.

35 16. The process according to claim 2, 14 or 15, wherein the microporous hollow fibre membrane substrate is made up of a mixture of polyethylenesulfide and polyvinylpyrrolidone having an inner diameter of 200-1000 μm , preferably about 330 μm , a wall thickness of 20-200 μm , preferably about 110 μm , a pore diameter of 0.1-0.8 μm , preferably about 0.4 μm , and is assembled in modules each

having 1 hollow fibre or bundles or modules of up to more than 1000 fibres.

17. The process according to claim 2 or any one of claims 14-16, wherein the ignition frequency during the plasma ignition is 1 kHz - 13.56 MHz or multiples of 13.56 MHz or microwave frequency, the power is 0.5-20 W, the voltage of the electrodes is 50-500 volts, the pressure is 0.01-10 mbar, the flow rate is 0.1-200 sccm/min, and the gas plasma mixture flow period is up to 20 min.

18. The process according to claim 2 or any one of claims 14-17, wherein the gas mixture is added to the housing or casing space surrounding the outer surface of the microporous hollow fibre membrane substrate in a diffusion controlled way at a pressure of 0.01-50 mbar.

19. The process according claim 2 or any one of claims 14-17, wherein the gas mixture is added to the housing or casing space surrounding the outer surface of the microporous hollow fibre membrane substrate in a laminar flow or convection controlled way at a pressure of 50 mbar-1.1 bar.

20. The process according to claim 2 or any one of claims 14-17, wherein the gas mixture is added to the lumen of the microporous hollow fibre membrane substrate in a laminar or convection controlled way at a pressure of 0.01-50 mbar.

21. The process according to claim 2 or any one of claims 14-17, wherein the gas mixture is added to the lumen of the microporous hollow fibre membrane substrate in a diffusion controlled way at a pressure of 50 mbar-1.1 bar, and wherein the housing space surrounding the outer surface of the microporous hollow fibre membrane substrate is filled with a blocking fluid, preferably polyethylene glycol.

22. The process according to any one of claims 3-13, wherein the microporous flat sheet membrane substrate throughout the process is enclosed in a housing or casing having a first and a second compartment separated from

each other by said membrane substrate, wherein the surface on the filtrate side of said membrane substrate is facing the first compartment and the surface of the blood side is facing the second compartment, and wherein the gas mixture is added to said first compartment and the functional groups during the plasma ignition in the presence of the gas mixture are bound to pore surfaces and the surface on the filtrate side of the microporous flat sheet membrane substrate.

23. The process according to claim 22, wherein the flow rate of the gas plasma mixture obtained by the plasma ignition is 1-100 sccm/min, preferably about 10 sccm/min.

24. The process according to claim 3, 22 or 23, wherein the microporous flat sheet membrane substrate is made up of a mixture of polyethersulfone and polyvinylpyrrolidone having a wall thickness of 20-200 μm , preferably about 110 μm , and a pore diameter of 0.1-0.8 μm , preferably about 0.4 μm .

25. The process according to claim 3 or any one of claims 22-24, wherein the ignition frequency during the plasma ignition is 1 kHz - 13.56 MHz or multiples of 13.56 MHz or microwave, the power is 1-20 W, preferably about 5 W, the voltage of the electrodes is 50-300 volts, the pressure is 0.1-5 mbar, preferably about 0.3 mbar, the flow rate is 1-100 sccm/min, preferably 10 sccm/min, and the gas plasma mixture flow period is up to 30 min, preferably about 5 min.

26. The process according to any one of the preceding claims, wherein excessive gas is evacuated from the housing or casing spaces after the plasma ignition.

27. A microporous affinity membrane produced according to any one of the preceding claims and having regioselective affinity for compounds in blood or other biologically active fluids to be removed during purification of blood or said fluids, wherein said membrane is provided

ed with functional groups, bound only to the pore surfaces.

28. The microporous affinity membrane according to claim 27, wherein the functional groups are amino groups.

5 29. The microporous affinity membrane according to claim 27, wherein the functional groups also are bound to the surfaces on the filtrate side.

30. The microporous affinity membrane according to any one of claims 27-29, wherein ligands having specificity for the components in blood or other biologically
10 active fluids to be removed are bound to the functional groups.

31. The microporous affinity membrane according to any one of claims 27-30, wherein it is a microporous
15 hollow fibre membrane or a microporous flat sheet membrane.

32. A microporous affinity membrane according to claim 30, wherein the ligands are proteins, peptides, amino acids, carboxylic acids, nucleotides, oligonucleo-
20 tides, antigens, or antibodies, and mixtures of two or more thereof.

33. An adsorption device containing the microporous affinity membrane according to any one of claims 27-32.

34. Use of a microporous affinity membrane according
25 to any one of claims 27-32 for therapeutic apheresis.

35. Use of a microporous affinity membrane according to claims 27-32 for diagnostic applications.

36. Use of a microporous affinity membrane according to claims 27-32 for drug development applications.

30 37. Use according to any one of claims 34-36, wherein blood constituents are not activated during said use.

ABSTRACT

A process for production of a microporous affinity
membrane having regioselective affinity for compounds in
5 blood or other biologically active fluids to be removed
during purification of blood or said fluids is disclosed,
as well as a microporous affinity membrane produced by
said process, an adsorption device containing such a
microporous affinity membrane, and use of such a micro-
10 porous affinity membrane.

15

20

25

Election for publication = Fig. 1

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080
1081
1082
1083
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093
1094
1095
1096
1097
1098
1099
1100
1101
1102
1103
1104
1105
1106
1107
1108
1109
1110
1111
1112
1113
1114
1115
1116
1117
1118
1119
1120
1121
1122
1123
1124
1125
1126
1127
1128
1129
1130
1131
1132
1133
1134
1135
1136
1137
1138
1139
1140
1141
1142
1143
1144
1145
1146
1147
1148
1149
1150
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180
1181
1182
1183
1184
1185
1186
1187
1188
1189
1190
1191
1192
1193
1194
1195
1196
1197
1198
1199
1200
1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239
1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260
1261
1262
1263
1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300
1301
1302
1303
1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318
1319
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380
1381
1382
1383
1384
1385
1386
1387
1388
1389
1390
1391
1392
1393
1394
1395
1396
1397
1398
1399
1400
1401
1402
1403
1404
1405
1406
1407
1408
1409
1410
1411
1412
1413
1414
1415
1416
1417
1418
1419
1420
1421
1422
1423
1424
1425
1426
1427
1428
1429
1430
1431
1432
1433
1434
1435
1436
1437
1438
1439
1440
1441
1442
1443
1444
1445
1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500
1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
1528
1529
1530
1531
1532
1533
1534
1535
1536
1537
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560
1561
1562
1563
1564
1565
1566
1567
1568
1569
1570
1571
1572
1573
1574
1575
1576
1577
1578
1579
1580
1581
1582
1583
1584
1585
1586
1587
1588
1589
1590
1591
1592
1593
1594
1595
1596
1597
1598
1599
1600
1601
1602
1603
1604
1605
1606
1607
1608
1609
1610
1611
1612
1613
1614
1615
1616
1617
1618
1619
1620
1621
1622
1623
1624
1625
1626
1627
1628
1629
1630
1631
1632
1633
1634
1635
1636
1637
1638
1639
1640
1641
1642
1643
1644
1645
1646
1647
1648
1649
1650
1651
1652
1653
1654
1655
1656
1657
1658
1659
1660
1661
1662
1663
1664
1665
1666
1667
1668
1669
1670
1671
1672
1673
1674
1675
1676
1677
1678
1679
1680
1681
1682
1683
1684
1685
1686
1687
1688
1689
1690
1691
1692
1693
1694
1695
1696
1697
1698
1699
1700
1701
1702
1703
1704
1705
1706
1707
1708
1709
1710
1711
1712
1713
1714
1715
1716
1717
1718
1719
1720
1721
1722
1723
1724
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740
1741
1742
1743
1744
1745
1746
1747
1748
1749
1750
1751
1752
1753
1754
1755
1756
1757
1758
1759
1760
1761
1762
1763
1764
1765
1766
1767
1768
1769
1770
1771
1772
1773
1774
1775
1776
1777
1778
1779
1780
1781
1782
1783
1784
1785
1786
1787
1788
1789
1790
1791
1792
1793
1794
1795
1796
1797
1798
1799
1800
1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829
1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860
1861
1862
1863
1864
1865

Fig 1

Membrane adsorption device with regioselectively functionalized microporous hollow fibre membranes

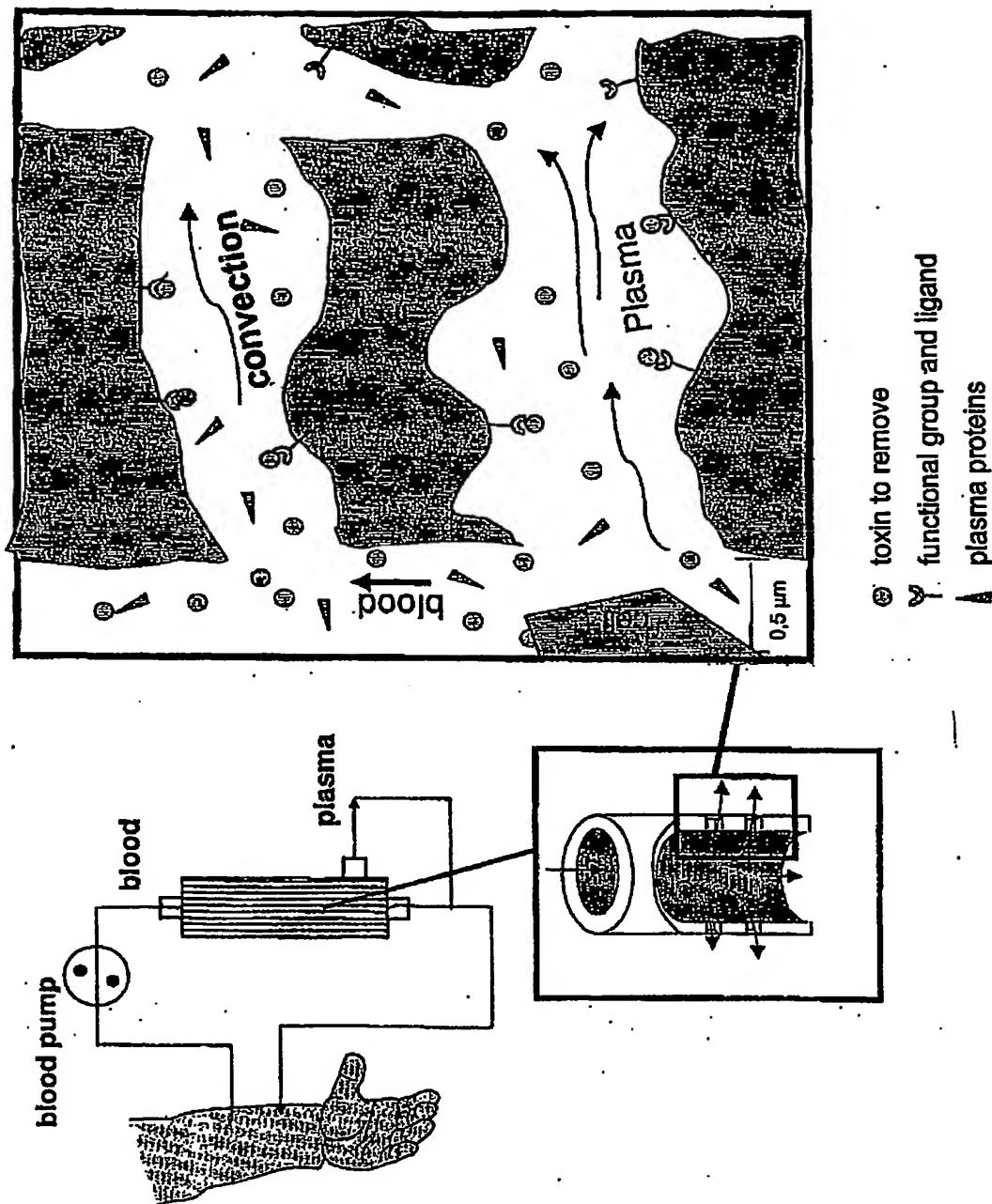
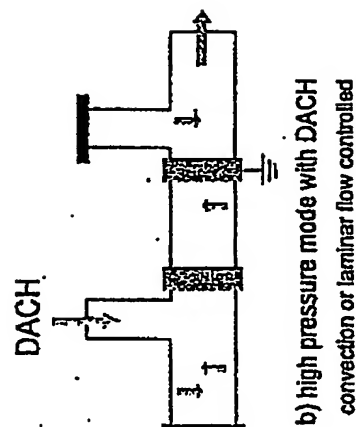
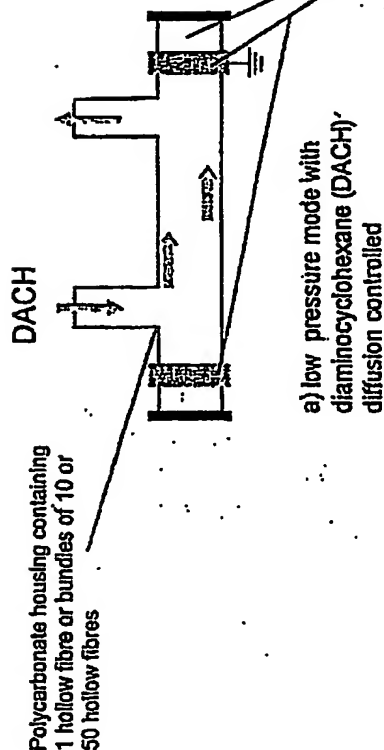


Fig 2

Plasma Treatment Modes

Outside-Plasma-Treatment



Inside-Plasma-Treatment

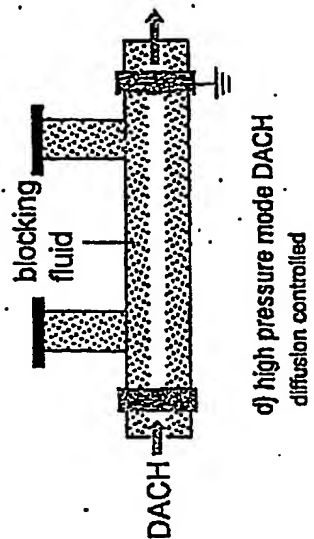
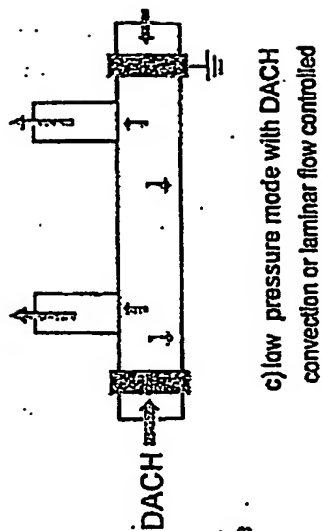


Fig 3

